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THE DIAGNOSIS OF BEE DISEASES BY LABORATORY METHODS

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INTRODUCTION.

Already some of the States have established laboratories for the diagnosis and investigation of the diseases of bees. The number of State laboratories where a rapid and accurate diagnosis of suspected material can be made will no doubt increase in the future in proportion as State laws for bee-disease inspection and control are instituted and perfected. Inspectors realize that there will be always some suspected brood and many conditions among adult bees which can not be diagnosed in the field, but which will require laboratory methods for diagnosis. In addition to the demands made by inspectors for the examination of suspected material, there will be numerous independent requests from beekeepers.

During the past several years the writers have examined a large number of specimens of suspected brood and bees sent to the Bureau of Entomology, and have developed and perfected methods and technique in the diagnosis of the known diseases of bees which, it is believed, will prove valuable to others. It is the aim of this paper to present these methods of diagnosis for the benefit of those who may engage in similar work.

For the understanding and application of the methods herein outlined, a preliminary training in general bacteriology, supplemented by a special knowledge of the pathogenic bacteria and the methods pursued in the diagnosis of diseases in general, is essential. As efficiency depends largely upon a knowledge of pathology, too much emphasis can not be given to it. With such general and special training as a basis, and with a knowledge of the bee diseases, the acquisition of the special methods necessary for a laboratory diagnosis of them becomes a comparatively simple matter.

One of the authors (White) began his investigations on the diseases of bees in 1902, and from this date to 1909 examined and diagnosed about 500 samples of suspected material and during the period developed the laboratory methods as given in the present paper. Since 1909 the senior author (McCray) has carried on the work of diagnosing the samples received by the Bureau of Entomology and up to the present time has examined and diagnosed about 5,000 of them.

LABORATORY METHODS.

OBTAINING THE SAMPLES.

For a satisfactory diagnosis of suspected brood the material at hand should be adequate. The size of the comb containing the affected larvæ should be ample. A piece about 5 inches square is suitable, provided it contains a sufficient number of larvæ. All samples, no matter how far sent or how long in transit, should be inclosed in wooden boxes to prevent crushing during transportation. Pasteboard boxes are apt to allow the sample to be crushed, while samples inclosed in tin boxes are often covered with a rich fungous growth when received. Both the crushing of the sample and the fungous growth obscure the gross features which form an important part of the examination. The sample should not be wrapped in cloth or paper, as this will tend to retain moisture and further encourage fungous growth. If thin paper is used, it frequently adheres so closely that its complete removal is difficult. The sample should contain no honey, as a small amount of it in a comb is sometimes sufficient to cause an unsightly mass, owing to leakage through the package in transit. As complete a history as can be obtained should accompany each suspected specimen as an aid to diagnosis. The Bureau of Entomology for several years past has sent out a printed question card with blank spaces for answers by the beekeeper sending the suspected brood. Some of the more important of these questions are as follows:

1. Is there any bee disease in your neighborhood?
2. If so, what disease?
3. Have you brought colonies from a distance? If so, give source.

4. Have you fed honey from other sources than your own apiary? Give source if known.
5. Is the diseased brood mostly capped or is it mostly uncapped?
6. What is the color of the larvæ (grubs) soon after death?
7. Later, what is the color of the decaying larvæ?
8. Are the dead larval remains ropy?
9. Do you notice any disagreeable odor in the hive?
10. Does there seem to be an unusual number of queenless colonies in the apiary?
11. What disease do you suspect?
12. Give location of the apiary from which sample was taken by town (or township) and county.

While a diagnosis sometimes can be made from larvæ which have been removed from their cells, and sent without the comb, such material is not satisfactory. It is far better to examine the infected larvæ in the comb in which they die. The diagnosis of bee diseases should not be based upon the examination of honey alone.

EXAMINATION OF THE SAMPLES.

In diagnosis both gross and microscopic examinations are made of the suspected material.

GROSS EXAMINATION.

The following points are to be taken into consideration: Character of the caps; regularity of the brood; proportion of affected brood; position of diseased larvæ within the cell; age, color, consistency, and odor of the affected brood; and kind of larvæ affected, whether queen, drone, or worker brood. These factors will be taken up in detail as each brood disease is considered separately.

In the gross examination of the comb it should be held in such a manner that a good lighting of the interior of the cells is secured. This is especially important in examining for scales. The best method of examining suspected brood is to hold the comb in a vertical position and about level with the eyes; then, by gradually inclining the top of the comb toward the observer, a point is reached at which the greatest amount of light is thrown upon the floor of the cells. This brings out the scales with great prominence and permits of their close scrutiny within the cells. In examining the affected brood for consistency and adherence to the cell walls in the scale stage, a small pair of curved forceps is convenient.

The gross examination of the adult bees will be considered under Nosema disease.

In routine diagnostic work unstained water mounts have been found very satisfactory in searching for spores, and stained preparations are made for the vegetative forms. Carbol fuchsin is a suitable stain to use.

MICROSCOPIC EXAMINATION.

The number of larvæ or pupæ to be examined in a given sample depends upon various factors. If the case is a typical one, one larva or pupa dead of a disease usually is sufficient. If, on the other hand, the gross appearance is not so definite and the microscopic picture from the first dead remains examined is unsatisfactory, others must be studied. While much might be written concerning the microscopic appearance of smears from larvæ or pupæ affected with the various brood diseases, as compared each with the others and with smears from healthy brood, such elaborate descriptions are not deemed advisable in this paper. Only the more salient features of the microscopic picture will be given for each disease considered, as it is believed that such descriptions will best serve those for whose benefit the paper is prepared.

It will be understood that when the authors write of the recognition of certain organisms by microscopic examination, as, for example, that of *Bacillus pluton* or *Bacillus alvei*, either in stained smears made from tissue or in stained smears made from agar plates, they refer to the recognition of the organism under observation only in a general way, meaning rather that the microscopic picture suggests the organism. The identification of the organism is complete, naturally, only after a consideration of its cultural characteristics also.

CULTURES.

In culturing the affected brood agar as ordinarily prepared in the laboratory is used in making plates. Those larvæ or pupæ are selected which upon microscopic examination have shown evidence of disease. Of course as many additional ones may be cultured as desired. The cultures are incubated for different periods of time, as will be noted in the discussion of the diseases.

Dead, not living, larvæ are examined.—In the laboratory examination of diseased bee brood, the affected larvæ are always received dead, therefore nothing will be said relative to symptoms and appearance of affected living larvæ.

DISEASES TO BE DIAGNOSED.

EUROPEAN FOULBROOD.

European foulbrood is an infectious disease of the brood of bees caused by *Bacillus pluton* (White, 1912).

GROSS CHARACTERS.

(*a*) *The caps and regularity of the brood.*—Larvæ that die of European foulbrood do so usually before they reach the age at which brood is capped. Brood dead of the disease is therefore usually found in uncapped cells. When the larvæ die after capping

the caps usually are entire, but may be punctured. The caps may be slightly sunken, but usually are not. Owing to the fact that such a small percentage of the affected brood becomes sealed, a comb of brood affected with European foulbrood with its few sealed cells and large amount of young uncapped brood presents a distinctive appearance (Pl. I, fig. 1) and shows a marked contrast to the solid areas of brood of uniform age in healthy combs.

(*b*) *Proportion of affected brood.*—European foulbrood usually has made rather extensive ravages by the time the beekeeper detects it; hence in many samples received for diagnosis a very large proportion of the larvæ in the comb are affected. Toward autumn, however, it is not unusual to receive samples containing a small number of affected larvæ.

(*c*) *Position of larvæ within the cell.*—The usual position for larvæ affected with European foulbrood is that of lying curled at the bottom of the cell. Other affected larvæ lie extended in the cell, but these are few in number.

(*d*) *Age of the dead larvæ.*—In most of the specimens received for diagnosis by far the larger proportion of the affected larvæ are young, lying curled at the bottom of the cell as just stated. Besides this comparatively young brood, older larvæ, including a few sealed ones, may be found affected.

(*e*) *Color.*—A change in color is one of the first abnormalities noted in brood dead of European foulbrood. Yellow or gray and combinations of these two colors are among the first to be noted. Later the yellow and gray gradually deepen, until quite a dark brown is attained. Larvæ dead of this disease often present a peculiar appearance, as though they were melting away under the influence of heat. The transverse tracheal branches stand out prominently. This melting appearance of the larvæ, the yellow, gray, and brown coloration, prominent tracheal branches, and large amount of uncapped affected brood are characteristics not easily confused, in the majority of cases, with those of other diseased conditions of the brood.

(*f*) *Consistency.*—Larvæ dead of European foulbrood are comparatively friable. However, larvæ which are somewhat viscid usually may be found. Perhaps slimy, rather than viscid, expresses better the consistency of some of these larvæ.

(*g*) *Odor.*—A slight, inoffensive odor is frequently to be noted in European foulbrood. The yeastlike odor which has been described is not constant in brood affected with European foulbrood. A similar odor may be detected in samples other than those which contain European foulbrood.

(*h*) *Kind of brood affected.*—Sometimes samples are received which contain only affected drone-brood. Most cases, however, consist only of worker-brood. Queen larvæ also may be attacked.

(*i*) *Scales.*—Scales are formed by the drying of the affected larvæ, and from the foregoing description of the dead brood some conception may be gained as to their form and appearance. Scales of European foulbrood, like most of the affected larvæ, are small and lie at the bottom of the cell, from which they can be separated with ease. The color of the scale is in general yellow, gray, or brown, and the cross markings formed by the transverse tracheal branches usually are still in evidence. This is the usual type of scale found in European foulbrood. Occasionally there will be received, however, a sample containing only a few scales, or perhaps a single scale, in marked contrast to the scale just described. These scales are always few in number in a given comb area, are usually dark brown in color, are less easily removed than the small ones, and are not brittle but rubberlike in consistency.

MICROSCOPIC FINDINGS.

The appearance of *Bacillus pluton*, the etiological factor in European foulbrood, in stained preparations usually is sufficiently characteristic to render its microscopic identification comparatively certain. Besides *Bacillus pluton*, the following secondary invaders may be found: *Bacillus alvei*, *Streptococcus apis*, *Bacillus vulgatus*, *Bacillus mesentericus*, *Bacillus orpheus*, and *Bacterium eurydice.* Without careful observation *Bacillus pluton* and *Streptococcus apis* might be confused. Upon careful examination it is found that *Bacillus pluton* presents considerable variation in size and morphology in the individual organisms. Some of them occur in the form of cocci, yet the general picture is that of an organism with more or less pointed ends. Thin smears should be made in order to obtain details of morphology.

It is important to have a true conception of the microscopic appearance of *Bacillus pluton.* The essential facts are the typical morphology and the manner of grouping of the individual organisms. The general shape of the group is often more or less circular, although numerous groups of more or less irregular form may be observed. Groups of varying shapes and sizes will be noted as successive fields are brought into view. It is the presence of these groups, containing a sufficient number of organisms with the pointed ends described, that serves to differentiate *Bacillus pluton* from *Streptococcus apis. Streptococcus apis* usually occurs in forms which are sufficiently coccuslike to lead to little or no hesitancy in differentiating it from *Bacillus pluton.* Forms which are sufficiently pointed to resemble *B. pluton* do occur, however, and if only a few are present in a field the differentiation of these species is not possible. By making a sufficient number of smears from a sufficient number of larvæ, forms

in abundance typical of either *B. pluton* or *Strep. apis* usually can be found. In stained smears of *Strep. apis* the organisms are found to be spread out over the whole field with no tendency to grouping as in the case of *B. pluton.* Sometimes in examining European foulbrood larvæ the microscopic picture shows practically nothing but *B. pluton.* More often, however, *Strep. apis*, *B. alvei*, and other rod forms are found. Some larvæ will disclose *B. alvei* alone, others *Strep. apis* alone, and still others, these two organisms without *B. pluton.* Continued search is sometimes necessary before larvæ are found revealing *B. pluton*, either alone or with one or more of the secondary invaders just mentioned. The authors have found *Bacillus pluton* in the small yellow, gray, and brown scales as well as in the soft melting larvæ. They are not prepared, however, to state the length of time that the organism persists in the dried state. Mention has been made of larger scales of rubberlike consistency which occur only occasionally and in small numbers in a given comb. Such scales always yield microscopically *Bacillus alvei* in abundance, and usually this organism alone. The microscopic appearance of *B. alvei* in the spore stage is rather characteristic, the spores practically always showing vestiges of the rods clinging to them. This aids in differentiating it from *B. vulgatus* and *B. mesentericus.* *Bacillus orpheus* may be recognized microscopically in the spore stage by the position of the spore in the rod, it being eccentrically placed. *Bacterium eurydice* is a small, slender organism which does not form spores.

CULTURES.

At the present writing no medium suitable for growing *Bacillus pluton* has been devised, hence agar plates made from European foulbrood larvæ show only the secondary invaders—*B. alvei*, *Strep. apis*, *B. vulgatus*, *B. mesentericus*, and *B. orpheus.* *Bacillus alvei* is encountered very frequently and is always secured on culturing larvæ in which the microscopic examination has revealed the presence of the organism. *Streptococcus apis* occurs occasionally. *Bacillus vulgatus* and *B. mesentericus* frequently are met, but usually in small numbers only. *Bacillus orpheus* in large numbers is occasionally encountered. *Bacterium eurydice*, as a rule, does not appear in the cultures. *Bacillus alvei* is the only organism occurring with any marked degree of frequency and in any great numbers on agar plates made from affected larvæ of any of the known infectious brood diseases of bees. Rarely do cultures from larvæ dead from any cause other than European foulbrood show the presence of this species.

The appearance of *B. alvei* on agar plates is rather characteristic. The colonies usually occur in abundance, often being innumerable.

When a few colonies are present there is seen, under low magnification, a granular center for each colony surrounded by numerous smaller but similar growths. There is little chance for error in the identification of *B. alvei*, assuming that the gross characters of the suspected material cultured had suggested European foulbrood and that the microscopic examination of the material had suggested the organism.

Cultures should be incubated until the second day in making a diagnosis of suspected European foulbrood material, since spores of *B. alvei* are not produced in abundance until that time. Two days, then, is the minimum time in which a report can be rendered on this disease. *B. vulgatus*, *B. mesentericus*, and *B. orpheus* may be recognized, when present, by their morphology and cultural characteristics (McCray, 1917).

AMERICAN FOULBROOD.

American foulbrood is an infectious disease of the brood of bees caused by *Bacillus larvae* (White, 1907).

GROSS CHARACTERS.

(*a*) *The caps and regularity of the brood.*—A large amount of the affected brood is capped, and many of the caps may be sunken and many perforated. The coloration, the sunken and perforated caps, and the irregularity produced by the capped and uncapped cells present quite a characteristic appearance (Pl. I, fig. 2).

(*b*) *Proportion of affected brood.*—The proportion of affected to healthy brood in American foulbrood is, as a rule, high, although specimens secured early in the attack may show a considerable proportion of unaffected brood.

(*c*) *Position of the larvæ within the cell.*—Inasmuch as most of the larvæ in American foulbrood die after the time of capping, the position of the larvæ is that of extension along the floor of the cell. But the dead larvæ quickly lose their form and symmetry, so that a dark, shapeless mass soon occupies the lower portion and bottom of the cell.

(*d*) *Age of the affected larvæ.*—The usual age at which the larvæ are found dead of American foulbrood is just after the time of sealing. This fact is of importance in considering the size of the scale and its position within the cell. Rarely is young unsealed brood found affected in this disease.

(*e*) *Color.*—Most of the dead larvæ when received for diagnosis will be of a dark chocolate color. Only rarely are larvæ of the lighter shades of brown seen. The late stages of decay are very dark brown.

(*f*) *Consistency.*—The consistency of the affected larvæ is characteristic and pathognomonic. The larvæ are strikingly viscid, so

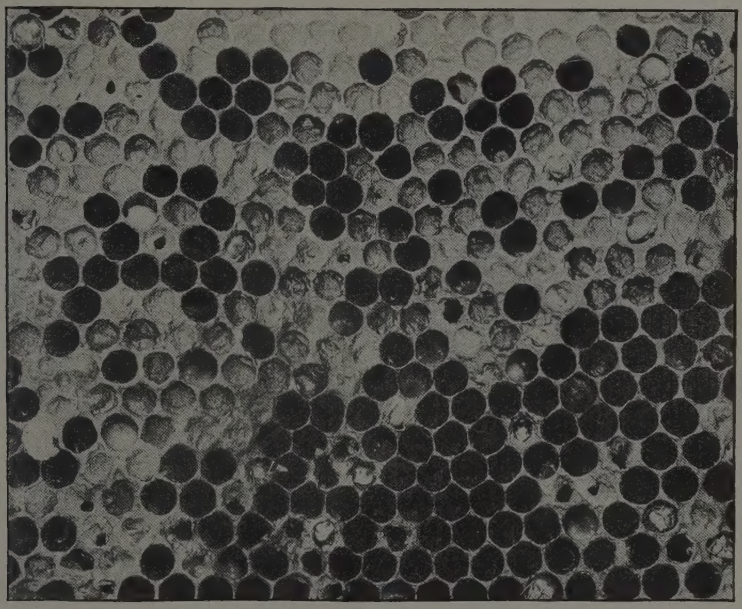

FIG 1.—COMB CONTAINING LARVÆ DEAD OF EUROPEAN FOULBROOD.
About natural size. (Original.)

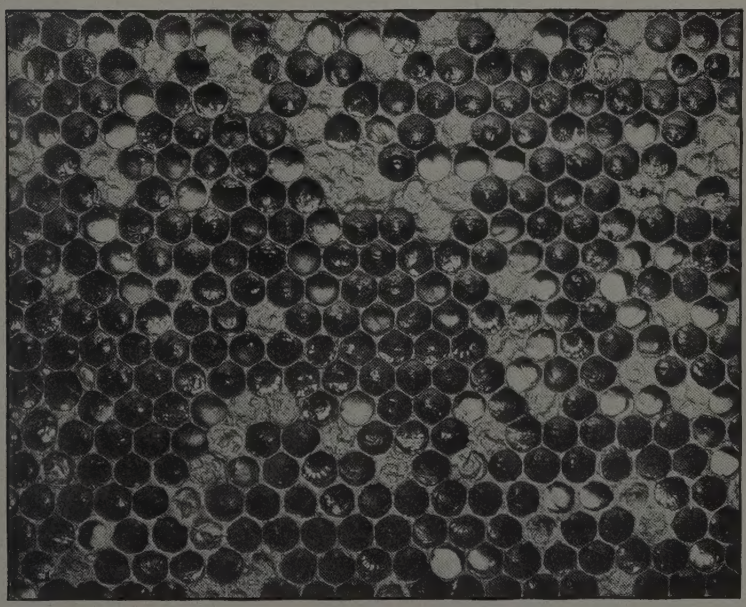

FIG. 2.—COMB CONTAINING LARVÆ DEAD OF AMERICAN FOULBROOD.
About natural size. (Original.)

EUROPEAN AND AMERICAN FOULBROOD.

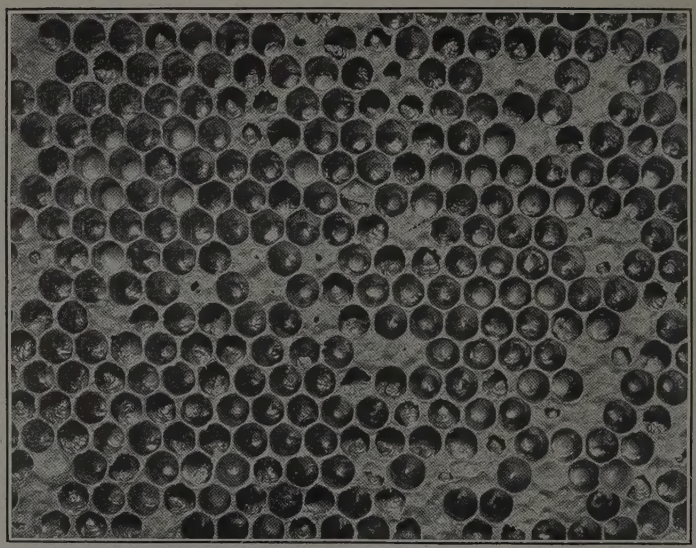

FIG. 1.—COMB CONTAINING LARVÆ DEAD OF SACBROOD.
Natural size. (White.)

FIG. 2.—STAINED SMEAR PREPARATION SHOWING SPORES OF NOSEMA APIS.
Highly magnified. (Original.)

SACBROOD AND NOSEMA SPORES.

that on thrusting the forceps into the brown larval remains and withdrawing them a portion of the decaying mass adheres and is drawn out, often to a distance of 3 or 4 inches. The viscidity is often referred to by the term "ropiness" in beekeeping literature. In the rare instances in which young uncapped affected larvæ are encountered the ropiness is less pronounced.

(*g*) *Odor.*—The odor is characteristic and may be described as unpleasant. Often it is feeble or absent altogether, probably having disappeared after the removal of the diseased brood from the hive. Affected brood-comb will absorb other odors if given the opportunity, thus masking the original characteristic odor. Such disappearance and masking of the odor has been observed where specimens of diseased brood in combs from various sources had been thrown together and allowed to lie about preparatory to being destroyed (McCray, 1916).

(*h*) *Kind of brood.*—It is affected worker-brood that is most often encountered in American foulbrood samples, although drone-brood is sometimes affected.

(*i*) *Scales.*—The scales of American foulbrood are distinctive and are characteristic of the disease. If they are present in sufficient numbers the disease can be diagnosed from the gross appearance alone. The lower cell walls can be easily illumined by tilting the upper portion of the comb toward the observer, as previously described. The scales appear extended along the lower cell wall, are quite dark in color, and adhere closely to the floor and base of the cell. Sometimes they adhere so closely as to break when an attempt is made to remove them from the cell. Often a semblance of the form of the pupæ is evident in the dried-down mass forming the scale. Some of the mouth parts of the pupæ sometimes protrude sufficiently to adhere to the roof of the cell.

MICROSCOPIC FINDINGS.

In considering the microscopic appearance of stained smears from infected brood in this disease, there usually is only *Bacillus larvae* in the spore form to engage the attention of the observer. In some instances, however, samples containing the disease in its earlier stages are received and then *Bacillus larvae* in the rod or vegetative form may be encountered. Such a sample usually is more difficult to diagnose. To receive a sample in which some older larvæ containing spores of *Bacillus larvae* can not be found, however, is a very rare occurrence.

In a stained smear made from an infected larva and mounted in water, the microscopic picture is rather characteristic. The most striking feature of the mount is the large number of spores adhering to the cover glass, floating with the current, or dancing free in

the water medium. Many of the spores stain slightly about the periphery, which aids somewhat in the observation. Good results may be obtained from an unstained water mount. The vegetative forms of *Bacillus larvae*, when they are present, are observed to be slender rods, which tend to occur in chains.

CULTURES.

In culturing affected larvæ it is the absence of growth on the agar plates that is important in the diagnosis of American foulbrood. This is because the spores of *Bacillus larvae* will not germinate and grow on the ordinary media of the laboratory, and other growth is absent because there are seldom secondary invaders present. Occasionally there will be a spreading growth of *B. vulgatus*, or *B. mesentericus*, and very rarely of *B. alvei*. On quite rare occasions a considerable number of colonies of *B. vulgatus* or *B. mesentericus* have been found. As both of these species form spores, as a rule, within 24 hours, their differentiation from *B. alvei* usually can be made in this way. A report on a sample of American foulbrood, therefore, nearly always can be made within a day.

SACBROOD.

Sacbrood is an infectious brood disease of bees caused by a filterable virus (White, 1913 and 1917).

GROSS CHARACTERS.

(*a*) *Character of caps and regularity of the brood.*—Larvæ usually die after capping in this disease, some of the dead brood being uncapped by the bees later. Occasionally the caps are punctured. An area of comb affected with sacbrood therefore presents an irregularity. So far as the age of the affected larvæ themselves is concerned, there is considerable uniformity owing to the fact that death in this disease occurs after sealing during the two-day period of rest just preceding pupation. The affected brood, however, is interspersed among healthy brood of varying age, which adds to the irregular appearance of the affected comb (Pl. II, fig. 1).

(*b*) *Proportion of affected brood.*—As a rule there is not a large proportion of affected brood in a given comb area. Often there will be an affected larva only here and there.

(*c*) *Position within the cell.*—The position of the affected larvæ is that of extension lengthwise along the floor of the cell, against which the dorsal portion of the larva lies. The head is turned upward, toward the roof of the cell.

(*d*) *Age.*—The brood dies after it has been sealed.

(*e*) *Color.*—Usually by the time brood is received for diagnosis the color of the affected larvæ is brown or quite dark—often almost black. If the brood is in the earlier stages of decay, however, the

color may be light yellow, light gray, or light brown. The lighter shades soon deepen to the darker ones.

(*f*) *Consistency.*—The consistency is characteristic. The cuticular portion of the body wall of an affected larva is decidedly resistant so that the larva may be grasped with forceps and removed from the cell intact. After removal from the cell the larva has the appearance of a small closed sac. When the sac is ruptured the contents will be seen to be watery. Suspended in the waterlike fluid will be noted numerous fine brown granules.

(*g*) *Odor.*—There is no distinctive odor to sacbrood combs.

(*h*) *Kind of brood.*—The greatest ravages occur in the worker-brood. Affected drone-brood may be encountered.

(*i*) *Scales.*—The scales when dried down are quite black and the surface appears somewhat roughened. They separate readily from the cell wall and may be lifted out intact by means of forceps.

MICROSCOPIC FINDINGS.

The striking feature of the microscopic examination is the absence of microorganisms. Rarely a few rods may be observed. A large amount of detritus is always in evidence, consisting of the brown granular material seen on gross examination after rupturing the body wall of the larva. These granules are in a large part the result of the disintegration of the fat body of the larva.

CULTURES.

As might be expected from the microscopic examination, agar plates inoculated with infected material are practically always negative as to bacterial growth. Even the presence of organisms of the *vulgatus* group is rare. No other growth occurs unless from chance contamination.

OTHER ABNORMAL CONDITIONS OF THE BROOD.

There are noninfectious abnormal conditions of the brood of bees which have been confused with one or more of the infectious diseases. Among the more important of these may be mentioned chilled brood, starved brood, overheated brood, drone-brood resulting from laying workers, and brood dying after removal from the hive. The names given to most of the foregoing conditions are sufficient to indicate in a general way the probable cause of death. These conditions are less likely to be confused with American foulbrood than with either one of the other two infectious brood diseases of bees. The specimens that resemble European foulbrood in the gross consist of soft, easily ruptured, gray, yellow, and light-brown larvæ. The irregularity of the brood, the age, the color, and sometimes the scales bear a striking resemblance to many cases of the disease.

On microscopical examination of the affected larvæ the smear often discloses microorganisms, yet they lack the definite, clear-cut,

positive picture desired in the diagnosis of the disease. If, on culturing, the agar plates are free from *Bacillus alvei* the specimen is usually considered negative.

Occasionally specimens are received resembling sacbrood that tend to cause confusion. The head in some of these has a tendency to turn upward, resembling sacbrood, but unless there is present the tough body wall and watery granular contents, a diagnosis of sacbrood should not be made. A resemblance to the disease is sometimes noted after the brood dries down to form a scale, dark in color and separating readily from the cell wall. The microscopic examination and the cultures are often negative as in sacbrood. In such cases, when there are only a few affected larvæ, it is impossible to make a diagnosis. Samples of comb containing only pollen without brood or scales have been received for diagnosis. Such specimens are always unsatisfactory and insufficient for diagnosis.

TABLE 1.—*Differential features in the diagnosis of the brood diseases of bees by laboratory methods.*

	European foulbrood.	American foulbrood.	Sacbrood.
General appearance of brood.	Brood irregular. Large amount of affected brood unsealed.	Very irregular; affected brood sealed, sunken and perforated caps present.	Brood less irregular, perforated caps present, dark sunken caps not so pronounced as in American foulbrood.
Proportion of affected brood.	Varying number of young larvæ affected, usually many.	Usually a large amount of brood affected.	Small amount of brood affected.
Position within cell	Usually curled at bottom. Larvæ soft, with melting appearance.	Extension along lower cell wall. Larvæ soon become a shapeless mass.	Extension along lower cell wall. Head turned upward. Normal form maintained.
Age of the larvæ	Usually die before capping.	Usually die after capping	Almost invariably die after capping.
Coloration	Larvæ yellow, gray, and brown.	Usually dark chocolate.	Soon become dark brown to almost black.
Odor	Slight, inoffensive.	Usually strong characteristic odor. More or less offensive.	None.
Consistency	Soft, rather friable.	Viscid, can be "roped" out a distance of 3 or 4 inches.	Contents watery and granular. Larvæ can be removed from cell without rupturing body wall.
Kind of brood affected	Often considerable amount of drone-brood as well as worker-brood.	Any considerable amount of drone-brood less likely to be seen.	Greatest ravages among worker-brood.
Scales	Usually small and lie at bottom of cell. Yellow, gray, or brown in color. Sometimes a few larger, brown, rubberlike scales. All scales separate readily from cell wall.	Extension along lower cell wall dark brown in color. Surfaces somewhat smooth. Separate from cell with difficulty.	Extension along lower cell wall. Dark in color, often black. Somewhat roughened appearance. Separate readily from cell wall.
Microscopic findings	*Bacillus pluton* always. *Bacillus alvei* usually. *Streptococcus apis* sometimes. *Bacillus orpheus*, *Bacterium eurydice*, *Bacillus vulgatus*, and *Bacillus mesentericus*, occasionally.	Usually only *Bacillus larvæ*. Occasionally *Bacillus vulgatus* and *Bacillus mesentericus*.	Negative as a rule.
Cultures	Any of the above organisms except *Bacillus pluton*.	Frequently negative. Never *Bacillus larvæ* on common media.	Nearly always wholly negative.

NOSEMA DISEASE.

Nosema disease is an infectious disease of adult bees. It is the only adult disease which at the present time can be diagnosed by laboratory methods (White, 1918). Sixty years ago Dönhoff (1857) observed an infectious condition among adult bees in which, upon examining the stomach of affected bees, small oval bodies were found. This work had been practically forgotten until Zander (1909) reported some interesting findings in a disorder of adult bees. He found that the stomach wall of bees taken from colonies suffering from what he called "malignant dysentery" contained a protozoan parasite. To this parasite he gave the name *Nosema apis.*

In England (Graham-Smith, Fantham, Porter, Bullamore, and Malden, 1912) Nosema infection in bees has been associated with a disorder referred to as the Isle of Wight bee disease. Recent investigations in Scotland (Anderson and Rennie, 1916) have lead to a somewhat different view. As *Nosema apis* occurs in the group Microsporidia the name "microsporidiosis" has been given to the disease (Fantham and Porter, 1912).

Nosema disease is widely distributed. It occurs in Germany, Australia, Switzerland, and England at least. The junior author (White, 1914) has found the parasite *Nosema apis* in samples of bees from a large number of the States of the United States and from Canada. The disease weakens and even kills colonies and is therefore one of interest to beekeepers. The exact losses from it are not known, but in America they are less than has been attributed to it in some other countries.

OBTAINING THE BEES.

Either dead or living bees are suitable for examination. Dead bees may be dry and still be suitable material. Living bees for examination can be sent very satisfactorily in mailing cages such as are used by queen breeders; dead ones may be sent in any convenient way. A complete history of the colony and apiary as to disease should accompany the bees.

GROSS CHARACTERS.

The presence of various symptoms has been mentioned as being of importance in the diagnosis of Nosema infection. Among these are noted the spotting of the hive with feces, abdominal distention, the presence of shiny bees devoid of hair, and the activity of the bees, either in the cages or when free. These are of questionable value. It is upon the presence or absence of Nosema spores that the diagnosis is based. Bees otherwise apparently healthy may, upon examination of the stomach, show the presence of spores of *Nosema apis* in large numbers.

MICROSCOPIC FINDINGS.

The bees if alive may be killed easily by crushing the thorax between the jaws of a pair of dissecting forceps. Then the thorax is grasped by the thumb and finger of one hand, the tip of the abdomen is grasped with a pair of forceps held in the other, and by gentle traction the ventriculus (stomach) and hind gut usually come away entirely and may be teased apart for examination, or the whole gut may be crushed under a cover glass and examined. In making a diagnosis at least 10 bees should be examined. Spores of *Nosema apis* if present are easily recognized, being oval, highly refractile bodies (Pl. II, fig. 2). Usually they occur in large numbers crowding the field. They stain with difficulty, and for diagnostic purposes water mounts unstained are satisfactory. The young forms of the parasite when present are quite difficult of detection, and should not be depended upon in the diagnosis.

Occasionally protozoa other than *Nosema apis* have been encountered in the examination of adult bees. These have no relation to Nosema disease, however, and may be disregarded in its diagnosis.

LITERATURE.

Much has already been written on bee diseases. The journals on beekeeping contain numerous articles pertaining to them. Bulletin No. 98 of the Bureau of Entomology briefly reviews a number of papers, published prior to 1912, dealing with the causes of these diseases. The papers reviewed and the publications cited, together with the papers in the following list and the references which they contain, comprise a fairly comprehensive résumé of all the literature detailing work done on these diseases.

With regard to further papers to appear soon, it is announced that studies have been made on American foulbrood and European foulbrood similar to those on sacbrood (White, 1917) and Nosema disease (White, 1918) and that the results are now being prepared for publication.

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